Aromatic profiles of trunk, limb, and leaf essential oils of *Juniperus scopulorum* (Cupressaceae) from Utah

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ABSTRACT

Volatile oils produced through steam distillation of *Juniperus scopulorum* were examined to establish essential oil yields and aromatic profiles from each portion of the tree, namely the trunk, limb, and leaf. The resulting essential oils were examined by GC-FID and GC-MS. Each plant portion exhibited a distinct aromatic profile. Trunk essential oil was prominent in cis-thujopsene, α-pinene, cedrol, allo-aromadendrene epoxide, (E)-caryophyllene, and widdrol, which averaged over all samples were 34.2%, 20.5%, 18.9%, 4.3%, 2.4%, and 2.4%, respectively. Limb essential oil was primarily α-pinene (average 82.4%) and leaf essential oil was primarily sabinene (average 66.9%). Complete profiles and yields are reported. *Published online www.phytologia.org Phytologia 103(1): 10-17 (March 22, 2021). ISSN 030319430.*

KEY WORDS: *Juniperus scopulorum*, Cupressaceae, aromatic profile, essential oil, trunk, limb, leaf, yield.

The genus *Juniperus* consists of nearly 75 species (Adams and Schwarzbach 2013). *Juniperus scopulorum* Sarg., commonly called Rocky Mountain juniper, is generally a montane species that has a wide distribution throughout many states in the intermountain region of the United States (Cronquist et al. 1972) and can be found in every county in the state of Utah (Welsh 1993). *J. scopulorum* is a dioecious species, though little variation in the essential oil profile of male and female trees has been observed (Adams and Powell 1976). Adams (2015) found that allopathic hybridization can occur between *J. scopulorum* and *J. maritima* in certain regions. However, Adams' genetic study did not show this hybridization in Utah populations of *J. scopulorum* analyzed.

The heartwood of *J. scopulorum* is red to purple in color and highly aromatic (Cronquist et al. 1972; Welsh 1993). The essential oil profile for *J. scopulorum* heartwood has previously been established as being prominent in cis-thujopsene (Adams 1987). Leaf oil is prominent in sabinene (Adams and Hagerman 1976, 1977; Adams 2009; Zheljazkov et al. 2013, 2017) and exhibits a significant degree of variation in its aromatic profile. These differences are associated with various factors including diurnal variation (Adams and Hagerman 1977), seasonal variation (Powell and Adams 1973; Adams 1979), maturity and age of the leaf material (Adams and Hagerman 1976), and distillation time (Zheljazkov et al. 2013).

J. scopulorum was a valuable medicinal plant among the Native Americans. The Blackfeet used an herbal tea made from the berries to deter vomiting, and the Cheyenne made an herbal tea from its leaves to treat both cough symptoms and as a sedative for hyperactive persons (Kindscher 1992).

To the authors' knowledge, the present study on *J. scopulorum* from Utah is the first to establish the complete aromatic profile for trunk and limb essential oils, and it confirms previously established aromatic profiles for leaf essential oils. Essential oil yields for each portion of the tree are also examined.

MATERIALS AND METHODS

Juniperus scopulorum plant material was collected from privately owned land in Duchesne County, Utah. Trees (n=3) were cut approximately 20 cm above ground and each tree was meticulously divided into three sections, namely the trunk, limbs, and leaf, to determine weight, yield, and aromatic profile of each portion of the tree. The trunk is defined as limb/leafless sections including heartwood, sapwood, cambium, and bark. The limb is defined as leafless, 1-5 cm diameter sections nearest the trunk. The leaf is defined as green foliage with minimal woody material, often including small berries. Collection details are recorded in Table 1. Voucher samples which were used for identification are held in the Utah Valley University Herbarium (UVSC): *J. scopulorum* Sarg., Wilson 2020-01, -02, -03 (UVSC).

Table 1. Collection details for each individual tree.

	***Juniperus scopulorum* #1**	***Juniperus scopulorum* #2**	***Juniperus scopulorum* #3**
date	19 May 2020	15 June 2020	21 July 2020
location	40°20’43”N 110°45’13”W	40°20’44”N 110°45’0”W	40°20’44”N 110°45’18”W
elevation (m)	2371	2365	2370
tree height (cm)	518	572	518
tree width (cm)	340	221	312

Portions of each tree were processed as follows for laboratory scale distillation: each mentioned portion of the tree was cut, chipped, bagged and stored at -20 ± 2 °C until it was steam distilled. Steam distillation was performed in triplicate, resulting in 9 distillations per tree and 27 distillations over the course of this project.

Laboratory scale distillation was as follows: 3 L of water added to the bottom of a 12 L distillation chamber (Albrigi Luigi S.R.L., Italy), plant material accurately weighed and added to the distillation chamber, distillation for 2 hours from pass-over by direct steam, essential oil separated by a cooled condenser and Florentine flask. Essential oil samples were filtered and stored in a sealed amber glass bottle until analysis.

Essential oils were analyzed, and volatile compounds identified by GC-MS using an Agilent 7890B GC/5977B MSD and J&W DB-5, 0.25 mm x 60 m, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of neat sample, 150:1 split ratio, initial oven temperature of 40 °C with an initial hold time of 5 minutes, oven ramp rate of 4.5 °C per minute to 310 °C with a hold time of 5 minutes. The electron ionization energy was 70 eV, scan range 35–650 amu, scan rate 2.4 scans per second, source temperature 230 °C, and quadrupole temperature 150 °C. Volatile compounds were identified using the Adams volatile oil library (Adams 2007, pdf at www.juniperus.org) using Chemstation library search in conjunction with retention indices. Note that in all samples limonene/β-phellandrene and in some samples widdrol/cedrol elute as single peaks, but their amounts are determined by the ratio of masses 68 and 79 (limonene), 77 and 93 (β-phellandrene), 151 (widdrol), and 150 (cedrol). Volatile compounds were quantified and are reported as a relative area percent by GC-FID using an Agilent 7890B and J&W DB-5, 0.25 mm x 60 m, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (20% soln. for essential oils, 1% for reference compounds), 25:1 split ratio, initial oven temperature at 40 °C with an initial hold time of 2 minutes, oven ramp rate of 3.0 °C per minute to 250 °C with a hold time of 3 minutes. For quantification, compounds were identified using retention indices coupled with retention time data of reference compounds.

The percent yield was calculated as the ratio of mass of processed plant material immediately before distillation to the mass of essential oil produced, multiplied by 100.

RESULTS AND DISCUSSION

The aromatic profiles of the trunk, limbs, and leaf from three *J. scopulorum* trees are detailed in Table 2. Each reported value is an average from three samples distilled from that portion of the plant.

Table 2. Aromatic profile of *J. scopulorum* trunk, limbs, and leaf essential oil of three trees. Each reported value below represents the average of three essential oil samples distilled from each portion (trunk, limbs, leaves) of the same tree. Compounds detected in one but not all samples are denoted as not detected (nd). Values less than 0.1% are denoted as traces (t). Unidentified compounds less than 1.0% are not included. KI is the Kovat's Index using a linear calculation on DB-5 column (Adams 2007). Relative area percent is determined by GC-FID. Essential oil samples were analyzed in triplicate to ensure reproducibility (SD<1).

		Rocky Mountain Juniper Averages								
		Trunk (%)			Limbs (%)			Leaves (%)		
KI	**Compound:**	1	2	3	1	2	3	1	2	3
921	tricyclene	0.1	0.1	t	0.2	0.3	0.2	t	t	t
924	α-thujene	0.1	0.1	t	0.2	0.2	0.2	1.5	1.4	1.5
932	α-pinene	25.2	23.4	13.0	82.0	85.1	80.1	5.2	4.0	3.6
945	α-fenchene	0.1	t	0.1	0.2	0.2	0.3	0.1	0.1	0.1
946	camphene	0.2	0.2	0.1	0.6	0.6	0.5	0.1	0.1	0.1
969	sabinene	0.3	0.4	0.2	1.6	1.5	1.4	67.6	67.5	65.6
974	β-pinene	0.3	0.3	0.2	0.9	1.0	0.9	0.7	0.4	0.4
988	myrcene	0.4	0.4	0.2	1.6	1.6	1.4	0.6	0.2	0.9
1001	δ-2-carene	nd	nd	nd	t	nd	nd	0.3	0.1	nd
1002	α-phellandrene	t	t	t	t	t	t	0.1	0.1	0.1
1008	δ-3-carene	1.8	0.9	1.9	4.7	2.6	6.8	0.2	0.1	0.2
1014	α-terpinene	t	t	t	0.1	0.1	0.1	1.2	1.1	1.6
1020	p-cymene	t	t	t	0.1	0.2	0.1	1.0	1.6	2.1
1024	limonene	0.5	0.6	0.4	1.6	1.6	1.5	2.7	3.8	3.6
1025	β-phellandrene	t	t	t	0.2	0.1	0.1	0.2	0.1	0.1
1044	(E)-β-ocimene	t	nd	t	0.2	t	0.3	t	nd	0.1
1054	γ-terpinene	t	t	t	0.2	0.1	0.1	2.0	1.8	2.9
1065	cis-sabinene hydrate	t	nd	nd	t	t	t	0.6	0.4	0.5
1086	terpinolene	0.6	0.5	0.4	1.3	0.9	1.2	1.1	0.8	1.2
1098	trans-sabinene hydrate	t	t	t	t	t	t	0.2	t	0.2
1112	trans-thujone	t	t	t	nd	t	t	0.2	0.2	0.2
1118	cis-para-menth-2-en-ol	t	t	nd	nd	nd	nd	0.1	0.1	0.1
1136	trans-para-menth-2-en-ol	t	t	nd	nd	nd	nd	0.2	0.2	0.2
1140	trans-verbenol	t	0.1	0.1	t	t	t	0.1	0.1	0.1
1174	terpinene-4-ol	t	t	t	t	0.1	0.1	3.5	3.1	4.9
1179	p-cymene-8-ol	t	t	t	t	t	t	0.1	t	0.1

KI	Compound:	Trunk (%)			Limbs (%)			Leaves (%)		
1186	α-terpineol	t	t	t	0.1	t	t	0.1	0.1	0.1
1223	citronellol	t	nd	nd	t	nd	nd	0.1	0.1	0.1
1257	methyl citronellate	t	t	t	0.2	t	0.2	0.3	0.2	0.2
1274	pregeijerene B	nd	nd	nd	t	t	t	2.5	3.9	1.9
1282	(E)-anethole	0.1	t	t	t	t	t	0.3	t	0.1
1287	bornyl acetate	0.2	0.4	0.2	0.5	0.6	0.3	0.1	t	t
1326	iso-dihydro carveol acetate	t	t	t	nd	t	t	0.3	nd	nd
1345	α-cubebene	t	nd	nd	0.1	t	nd	t	nd	nd
1387	α-duprezianene	0.1	t	t	nd	nd	nd	nd	nd	nd
1389	iso-longifolene	0.1	0.1	t	nd	t	nd	nd	nd	nd
1413	β-funebrene	1.8	2.2	0.6	t	t	t	nd	t	t
1417	(E)-caryophyllene	2.0	2.1	3.2	0.7	0.9	1.8	0.4	0.4	0.7
1419	β-cedrene	1.1	0.8	1.1	t	t	t	0.2	0.2	0.1
1429	cis-thujopsene	41.0	34.1	27.4	2.1	1.7	1.2	nd	0.6	0.6
1436	isobazzanene	0.1	0.1	t	t	t	t	0.1	0.1	0.1
1449	α-himachalene	0.4	0.3	0.3	t	t	nd	0.1	0.2	0.1
1452	α-humulene	t	0.1	0.1	0.1	t	0.2	0.1	0.1	0.1
1498	pseudowiddrene	0.5	0.2	0.2	t	t	t	nd	t	nd
1500	β-himachalene	1.6	1.1	0.8	t	t	t	nd	t	0.1
1504	cuparene	1.3	1.7	1.6	t	t	t	nd	t	t
1514	β-curcumene	1.8	1.2	0.9	t	t	t	nd	t	t
1549	elemol	0.3	0.2	0.3	t	0.1	t	1.5	1.9	0.6
1582	caryophyllene oxide	0.3	0.3	0.3	t	t	0.2	nd	0.1	0.1
1589	allo-cedrol	0.4	0.6	0.6	t	t	nd	0.1	nd	nd
1599	widdrol	3.1	2.1	2.1	0.1	t	t	nd	0.3	0.1
1600	cedrol	7.5	16.5	32.8	0.3	0.3	0.4	nd	0.6	0.5
1668	14-hydroxy-9-epi-(E)-caryophyllene	0.6	0.4	0.6	t	t	t	nd	t	t
1685	α-bisabolol	0.6	0.7	0.7	t	t	t	nd	t	t
1639	allo-aromadendrene epoxide	3.2	5.3	4.5	t	0.1	0.1	nd	0.2	0.1
1708	thujopsenal	0.7	0.9	0.9	t	t	t	nd	t	t
1792	8- α-acetoxyelemol	t	t	t	t	t	t	1.0	1.7	1.1
2359	3- α-acetoxy manool	nd	nd	nd	t	t	t	1.0	1.0	0.8

Essential oil samples were analyzed in triplicate to ensure reproducibility (standard deviation (SD) < 1 for all compounds). The different portions contain distinct essential oil profiles. Yields are detailed in Table 3.

Table 3. Distribution of mass and essential oil (EO) yield averaged from samples from three *J. scopulorum* trees. Each tree was cut 20 cm above ground; all measurements and calculations are reflective of above ground portions.

		mass (g)	mass (%)	mass distilled (g)	yield EO (g)	yield EO (%)
Trunk	1	26239	34.4	1283.39	4.06	0.32
	2	27529	41.5	946.00	1.58	0.18
	3	22806	45.4	808.64	1.75	0.22
	Avg:	25525	40.4	1012.64	2.46	0.24
	%RSD (n=3)					30.05
Limbs	1	23720	31.1	2712.26	3.59	0.13
	2	11877	17.9	1511.95	1.70	0.11
	3	10257	20.4	1539.92	1.60	0.10
	Avg:	15285	23.1	1921.38	2.30	0.11
	%RSD (n=3)					13.48
Leaf	1	26210	34.4	1394.60	3.37	0.24
	2	26860	40.5	1430.15	1.61	0.11
	3	17149	34.2	1269.82	1.79	0.14
	Avg:	23406	36.4	1364.86	2.26	0.16
	%RSD (n=3)					41.67

Prominent compounds detected in the trunk essential oil of three *J. scopulorum* trees included cis-thujopsene (41.0%, 34.1%, 27.4%), α-pinene (25.2%, 23.4%, 13.0%), cedrol (7.5%, 16.5%, 32.8%), allo-aromadendrene epoxide (3.2%, 5.3%, 4.5%), (E)-caryophyllene (2.0%, 2.1%, 3.2%), and widdrol (3.1%, 2.1%, 2.1%). Cis-thujopsene was almost exclusively contained in the trunk essential oil, with a small percentage found in the branches, and only trace amounts detected in the leaf. Widdrol and cedrol are also prominent in the trunk essential oil compared to branch and leaf oil. Interestingly, the replicate samples of tree two showed considerable variation in α-pinene and cedrol. While the average value of α-pinene in the three trunk samples from tree two was similar to averages from trees one and three, α-pinene in the individual samples from tree two ranged from 8.6% to 46.9%. Variation of α-pinene in replicates from trees one and three, while pronounced, were much smaller with ranges of 14.8% to 31.5% and 7.3% to 21.7%, respectively. A similar result was found among the tree two replicates for both cis-thujopsene, which ranged from 24.5% to 40.3%, and cedrol, which ranged from 8.1% to 21.8%. These results are reported in Table 4. This variability within the same tree is difficult to explain since sampling and extraction were identical. Perhaps future research could consider whether samples were taken nearer the branches or the roots, rather than as an indiscriminate whole. Another possibility to examine is whether the percentage of the unique purple-red heartwood, compared to the amount of sapwood, cambium, or bark, in the distillation affects the essential oil composition. While all trunk samples contained some heartwood, it is possible the percentages in each were inconsistent, as the percentage of heartwood, sapwood, cambium, and bark was not distinguished in the sampling (Figure 1).

Table 4. Variability of α-pinene, cis-thujopsene, and cedrol in all replicate distillations of *J. scopulorum* trunk essential oil.

Trunk Essential Oil												
Tree:	1				2				3			
Replicate:	1	2	3	Avg	1	2	3	Avg	1	2	3	Avg
α-pinene	31.5	29.4	14.8	25.2	46.9	8.6	14.7	23.4	7.3	10.0	21.7	13.0
cis-thujopsene	38.4	38.1	46.5	41.0	24.5	40.3	37.4	34.1	30.0	29.2	23.0	27.4
cedrol	5.5	6.7	10.3	7.5	8.1	21.8	19.6	16.5	34.2	34.6	29.6	32.8

Interestingly, cis-thujopsene and cedrol were also found to be prominent aromatic compounds in the trunk essential oil of *Juniperus osteosperma,* a species habiting similar regions in Utah as *J. scopulorum*, albeit generally at different elevations (Adams 1987; Cronquist et al. 1972; Wilson et al. 2019). Further, α-pinene was found to be prominent in both the trunk and limb essential oils of *J. osteosperma* (Wilson et al. 2019).

Figure 1. Photo of *J. scopulorum* heartwood. All three trees contained purple-red heartwood.

Prominent compounds from *J. scopulorum* limb essential oil included α-pinene (82.0%, 85.1%, 80.1%), δ-3-carene (4.7%, 2.6%, 6.8%), and cis-thujopsene (2.1%, 1.7%, 1.2%). Of all three plant portions, α-pinene was by far detected in the highest concentration in the limb essential oil.

Prominent compounds from *J. scopulorum* leaf essential oil included sabinene (67.6%, 67.5%, 65.6%), α-pinene (5.2%, 4.0%, 3.6%), terpinene-4-ol (3.5%, 3.1%, 4.9%), pregeijerene B (2.5%, 3.9%, 1.9%), and γ-terpinene (2.0%, 1.8%, 2.9%). Sabinene is the predominant compound measured in the leaf oil and was detected in much lower amounts in trunk and limb oil. Pregeijerene B was unique to the leaf oil, as only trace amounts were identified in limb oil, and pregeijerene B was not detected in the trunk oil. Not surprisingly, the leaf oil was composed primarily of monoterpenes. However, two unique terpenoids, an oxygenated sesquiterpene and an oxygenated diterpene, were found in the leaf oil at greater than 1%, which were only measured in trace amounts in the trunk and limb oil: 8-α-acetoxyelemol and 3-α-acetoxy manool. This finding supports previous work examining the presence of pregeijerene B and 8-α-acetoxyelemol in the leaf essential oil of multiple *Juniperus* species (Adams 2004).

CONCLUSIONS

This study establishes both the aromatic profile of *J. scopulorum* limb essential oil as well as the complete profile of the trunk essential oil, not just the heartwood alone. The results of this study also confirm previous studies showing that cis-thujopsene is a primary constituent of *J. scopulorum* trunk essential oil (Adams, 1987) and that sabinene is prominent in the leaf essential oil (Adams and Hagerman 1976; Adams and Hagerman, 1977; Adams, 2009; Zheljazkov, et al. 2013; Zheljazkov, et al. 2017). However, our cis-thujopsene results are 20-30% lower than originally reported, likely because our sample was the entire trunk, not only heartwood (Adams, 1987). Our leaf oil also showed less variation than previously reported, likely due to our samples being harvested in the same season, same location, of similar maturity, and similar time of day. Our percent sabinene corresponded with the previously reported data about distillation time (Zheljazkov et al. 2013). The trunk and limb essential oils of *J. scopulorum* also contain similar profiles to those of *J. osteosperma*, while the leaf essential oils of both trees differ greatly from each other (Wilson et al. 2019).

Each plant portion of *J. scopulorum* has a distinct essential oil profile. Trunk essential oil is high in cis-thujopsene (34.2%), α-pinene (20.5%), and cedrol (18.9%). Limb essential oil contained on average 82.4% α-pinene. Leaf essential oil contained on average 66.9% sabinene. Yield was highest in the trunk essential oil, then leaf, then limbs, at 0.24%, 0.16%, and 0.11% respectively.

Future research will focus on determining the cause of the variability in the trunk essential oil within a single tree. Plans include sectioning the trunk into heartwood, sapwood, and bark for distillation and comparison, as well as comparing homogenized samples of the whole trunk to trunk samples taken near both branches and roots of the tree.

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